Comparison of alveolar and interstitial macrophage activation following exposure of rats to ozone

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Introduction

Alveolar macrophages (AM) are frequently studied because they are partly and readily collected by bronchoalveolar lavage (2)(7). Inversely, the functional characteristics of interstitial macrophages (IM), including interactions with other lung cell types under pathophysiological conditions, are essentially unknown because of their poor accessibility. Interstitial macrophages are in direct contact with matrix and other cells in the pulmonary connective tissue, and release of mediators or enzymes by these macrophages may have a greater effectiveness than if released by their sister cells in the alveolar space. The aim of this experiment was to evaluate some aspects of both alveolar and interstitial macrophages collected from rats exposed to ozone.

Material and methods

Animal and exposure : Sprague-Dawley male rats (IFFA CREDO, France) weighing 180-200 g were exposed to clean air or 0.4 ppm ozone (6 hrs/day, 5 days/week). in 2-m^3 Hazleton inhalation chambers and sacrificed after 2 or 10 days of exposure. Ozone was produced by electric discharges through an oxygen flux (laboratory ozonator type BA.023, Wallace et Tiernan Ltd).

Lung lavage and interstitial cell separation : Rats were anesthetized by intraperitoneal injection of 6% pentobarbital sodium (0.2 ml / 100 g). The lung was perfused via cannulation of the heart in order to discard the blood monocytes, and thus avoid further contamination of IM during processing of the lung. Then he was lavaged 18 times by instillation via the trachea of warm PBS without Ca^{2+} and Mg^{2+}, in order to collect free alveolar cells. The first lavage was discarded, the others were pooled and stored in ice.

The lung and trachea were removed from the thoracic cavity, and the trachea and major bronchi removed. The five lobes were cut in 500 μm thick slices, and sequential enzymatic digestion of the tissue with collagenase

and DNAse was carried out (4)(6). Interstitial and alveolar cells were purified by centrifugation on a percoll gradient. For alveolar cells, the top layer which consists mainly of dead cells and debris was discarded, in remaining fractions, macrophages comprised up to 95% of the cells. For interstitial cells preparations, only fractions 2 and 3 were collected, and these contained 80% of macrophages. Viability assessed by trypan blue dye staining was higher than 80%.

Macrophage cultures : Cells from different preparations were cultured in 6-well multiplates at a concentration of 3.10^6 macrophages/well in serum free medium (S-MEM, Gibco) containing 1% penicillin/streptomycin supplemented with 0.2% bovin serum albumin in a 5% CO_2 humid atmosphere at 37°C. After 18 hours of incubation, both alveolar and interstitial conditioned culture mediums were collected and stored frozen at -70°C until used for measurement of various mediators.

Cytokine dosages : Tumor necrosis factor-α (TNF-α) and interleukin 1-β (IL-1β) were measured in macrophage conditioned culture mediums using ELISA kits (Mouse TNF-α and IL-1β ELISA kits, Genzyme) as described by the supplier.

Assay for fibroblast growth factor : Growth factor activity was conducted as described previously (G. Lacroix, 1997).

Assay for matrix metalloproteases activities (MMPs) : The global activity of MMPs was evaluated using enzymography in gelatin-containing polyacrylamide gels (SDS-PAGE). The extent of proteolysis areas was evaluated by semi-automatic quantification of both the area and the intensity of lysis bands.

In addition, free gelatinolytic activity was measured by degradation of radiolabelled gelatin under non dissociative conditions (5). Results are expressed as mg hydrolyzed gelatin per 24hrs, per million macrophages.

Statistics : All results are expressed as mean ± standard deviation. Statistical significance between exposed and control groups was determined using Student's t test (two by two homogeneous variances) or Cochran's test (two by two heterogeneous variances).

Results

Cytokine dosages : Figure 1a shows that for control rats the TNF-α secretion by either alveolar or interstitial cells was similar. After exposure to 0.4 ppm ozone for 2 and 10 days, alveolar culture mediums from exposed

rats contained significantly more TNF than that from control rats. Conversely, TNF secretion in medium from interstitial cells was not different from control values at any time point of exposure.

Results for IL-1 assays were similar (figure 1b). Ozone exposure induced a marked increase in IL-1 alveolar cell production. The increase is more pronounced after two days. Interstitial cells exhibited a capacity to secrete IL-1 only after 10 days, no difference between control and exposed rats was observed before.

Assay for fibroblast growth factor : The growth factor production pattern following O_3 exposure was very different regarding the two cell preparations (figure 2). We observed a decrease in culture medium from alveolar macrophages, and an increase in those from interstitial macrophages when compared to their respective counterparts from control rats.

MMPs activities : The results related to the total gelatinase activities obtained by enzymography are shown in table 1. 92 kDa gelatinase activity measured in the culture medium becomes apparent after ozone exposure. This production was not time dependent. Preparations from interstitial cells exhibited three activities at 72, 92 and 120 kDa, which increased with time of exposure to O_3. The inhibition pattern of 72 and 92 kDa gelatinase demonstrated that they were metalloproteases (not shown). The 72 kDa gelatinase is probably of fibroblastic origin. More investigations should be required to defined the nature of the 120 kDa gelatinase activity as it is not inhibited by known inhibitors of metallo, serine and cystein proteases.

The net gelatinolytic activity measured by degradation of radiolabelled gelatin substrate clearly increased in culture medium from alveolar macrophages of exposed rats. In culture medium from interstitial cells, no significant changes were observed between control and exposed rats (data not shown).

Discussion

These results demonstrate that following in vivo exposure to ozone, the cells collected from either the lung parenchyma or airways exhibited different reaction patterns with regard to inflammatory mediators release such as cytokines, growth factors and also in production of enzymes involved in remodeling of the extracellular matrix.

AM likely play a role in inflammatory cell recruitment via IL-1 and TNF production, whereas that of IM products (fibroblast growth factor) seems to be more involved in matrix component alterations.

We provided evidence for an increased of production of Type IV collagenase/gelatinase by alveolar and interstitial macrophages during ozone exposure. Nevertheless, it appeared that interstitial macrophages were also able to produce together a sufficent pool of metalloproteinase inhibitors. These inhibitors probably interact with the proteases to repress gelatinase activities in the culture medium. In the lung, the close contact between macrophages and the matrix favour their interactions and therefore the biological significance of metalloprotease released by IM in the remodeling of the interstitial matrix.

Therefore, it is possible that some compounds secreted by macrophages may regulate the activity of others, leading to an inhibition of production or activity (1).

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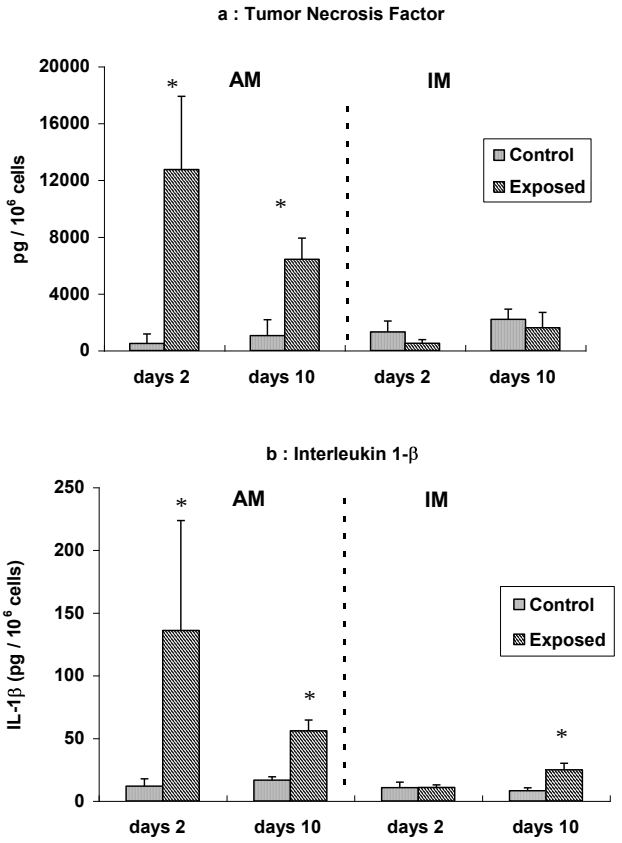

Fig.1. Cytokine secretion by alveolar (AM) and interstitial (IM) macrophages . Data shown are means ± SD for six rats per duration exposure. * $p<0.05$

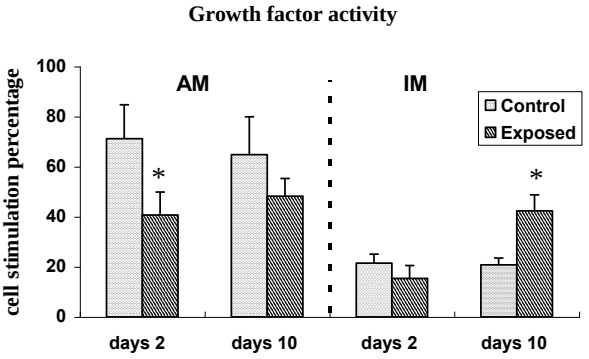

Fig.2. Growth factor activity for 3T3 fibroblasts in alveolar macrophage (AM) and interstitial macrophages (IM) conditioned mediums. Fibroblast growth stimulation by macrophage culture medium samples is expressed as the percentage of stimulation induced by 10% fetal calf serum (FCS). * $p<0.05$

table 1: Global activities of MMPs

	Control	0.4 ppm ozone	
		D2	D10
AM	-	92 kDa⮟	92 kDa⮟
IM	72 kDa 92 kDa 120 kDa	72 kDa 92 kDa⮟⮟ 120 kDa	72 kDa⮟ 92 kDa⮟ 120 kDa⮟